A PCR- Based Assay Using Sequence Characterized DNA Markers for the Identification and Detection of *Aphanomyces euteiches*

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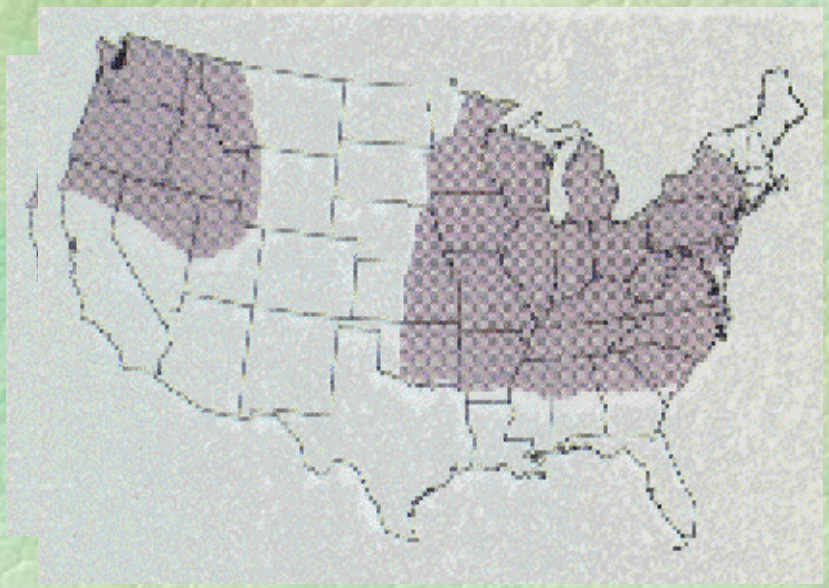
Aphanomyces euteiches

Plant pathogen that causes severe root rot disease in alfalfa, peas, and beans

Affected Regions



Infected alfalfa

USDA-ARS, Prosser, WA

Limitations of Conventional Methods for the Detection of *Aphanomyces*

- Use of selective media is confounded by presence of other fungicide resistant microbes i.e. *Pythium*.
- "Baiting" soil with susceptible host requires up to three weeks for completion.
- Microscopic detection of oospores is tedious and oospores are only produced at the end of season.

Soil Baiting Technique with Aphanomyces

OBJECTIVES

- Design a system based on PCR that could discriminate *A. euteiches* from other closely related species and genera of soilborne microbes.
- Use the system to detect *A. euteiches* in infected roots.
- Use the system to detect *A. euteiches* in soil.

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Advantages of PCR-Based Detection Assays for Soilborne Microbes

- Rapid
- Selectivity can be broad (genus) or narrow (species, race)
- Not necessary to isolate organisms in pure culture from soil/plant tissue
- Easier for ‘microscopically challenged’ pathologists

We chose to use SCARs for developing the assay

- SCARs = **S**equenced **C**haracterized **A**mplified **R**egions
- SCARs use a pair of sequenced characterized primers for amplification of target DNA
- SCARs were first developed for mapping resistance genes in lettuce to *Bremia lactucae*

Advantages of SCARs Over RAPDs

- Only detect a single locus (PCR product)
- Highly specific
- More rapid than RAPDs

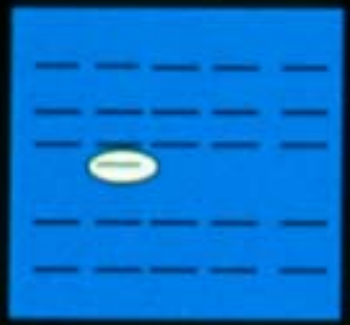
Method for Designing SCAR Primers

- Identify a RAPD that is only amplified by all isolates of the target species (*Aphanomyces* sp.)
- Clone and sequence the RAPD
- Design primers based on RAPD sequence
- Optimize thermocycling reaction conditions.
  - [$MgCl_2$] (1.5 mM-4.5 mM)
  - annealing temperature (60°C-72°C)

SCARs

RAPDs

PRIMER gcctacactg



CORTAR FRAGMENTO

PURIFICACION DEL FRAGMENTO DE ADN

CLONACION pcrII

SECUENCIACION DE EXTREMOS DEL FRAGMENTO DE ADN

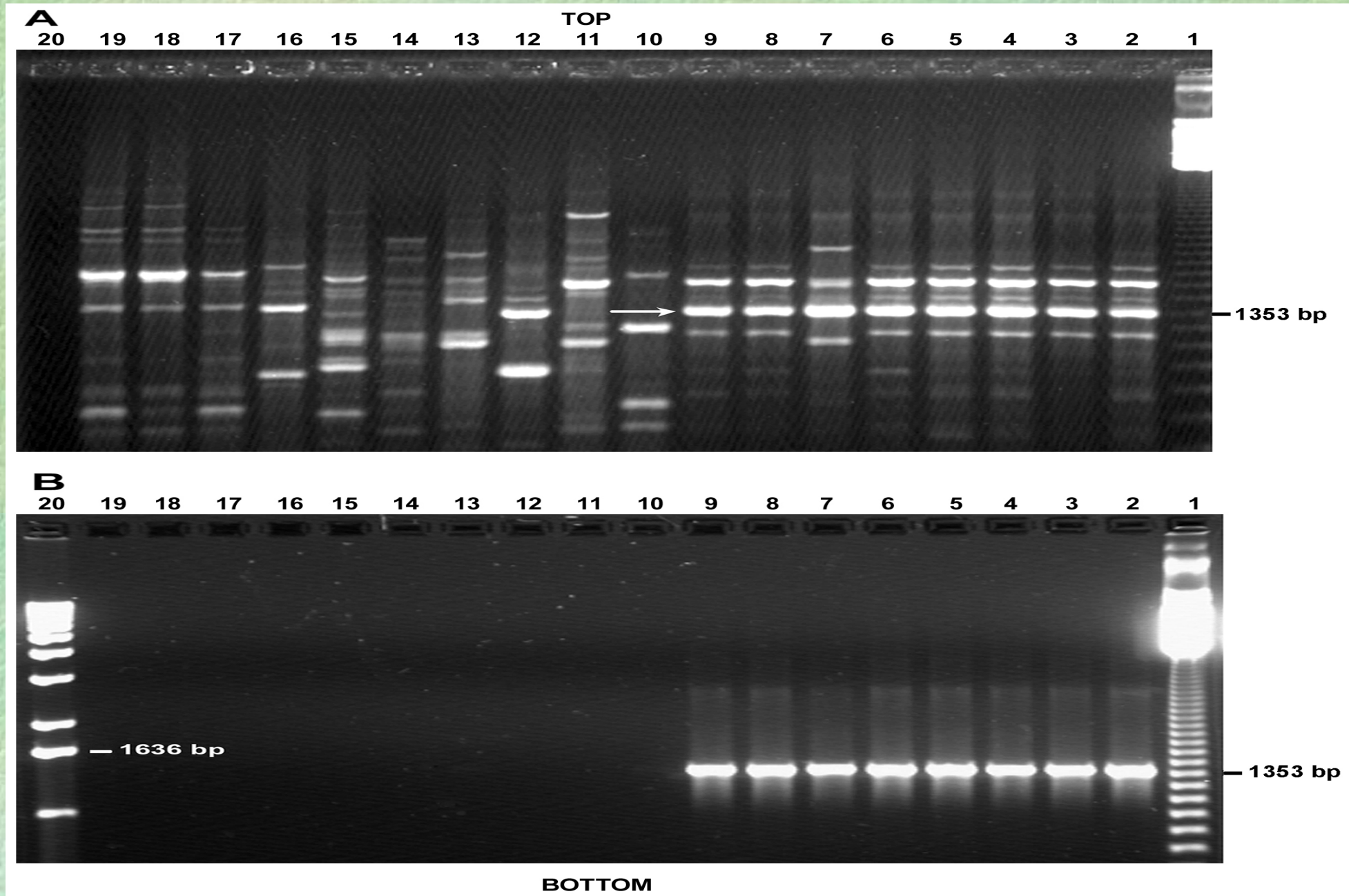
DISEÑO DE PRIMERS (20-25-mers)

gcctacactgTTCCATGCATTACGG

gcctacactgGACGTAAGCTGATT

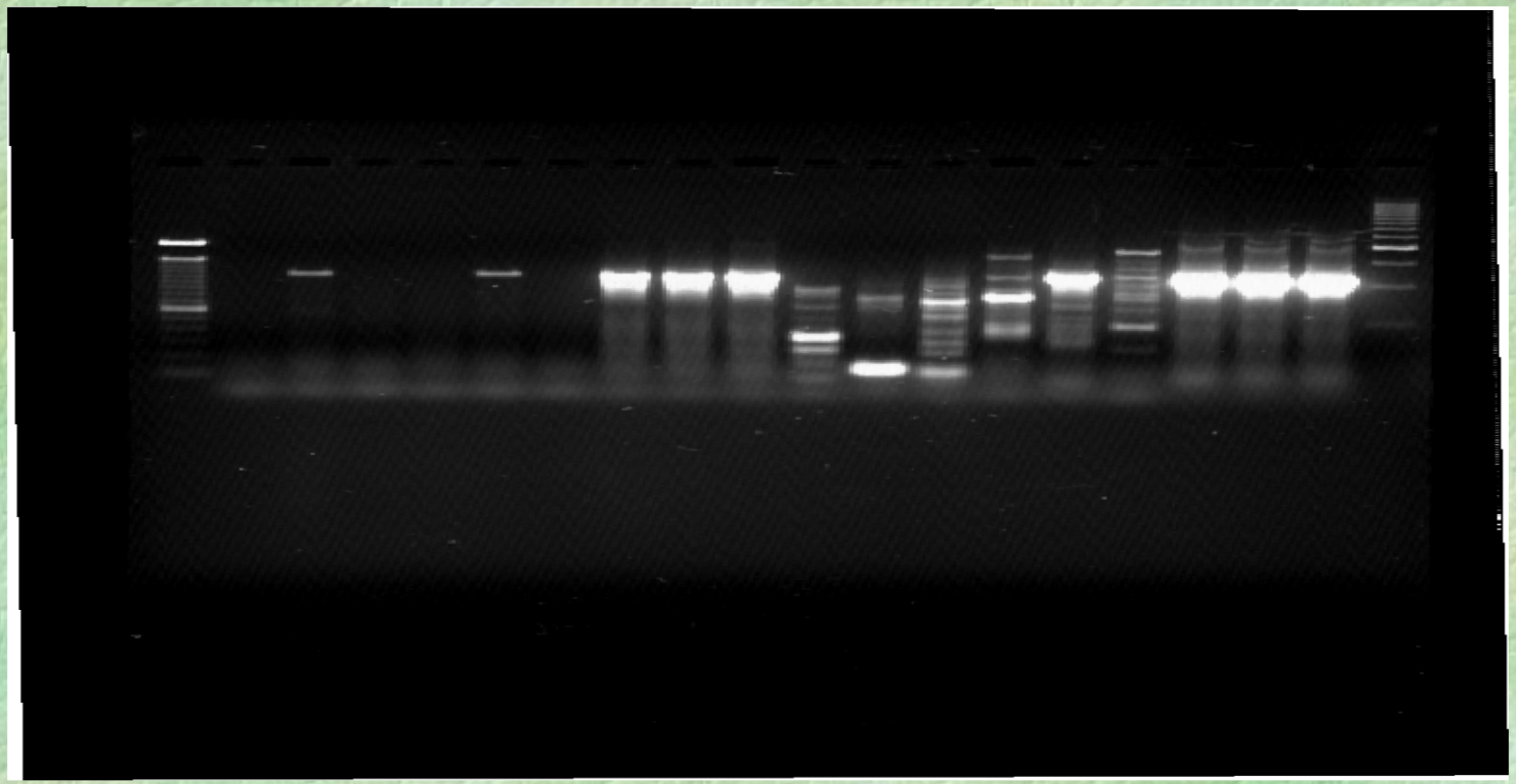
AMPLIFICACION DE ADN

Developing a SCAR Specific for *A. euteiches*



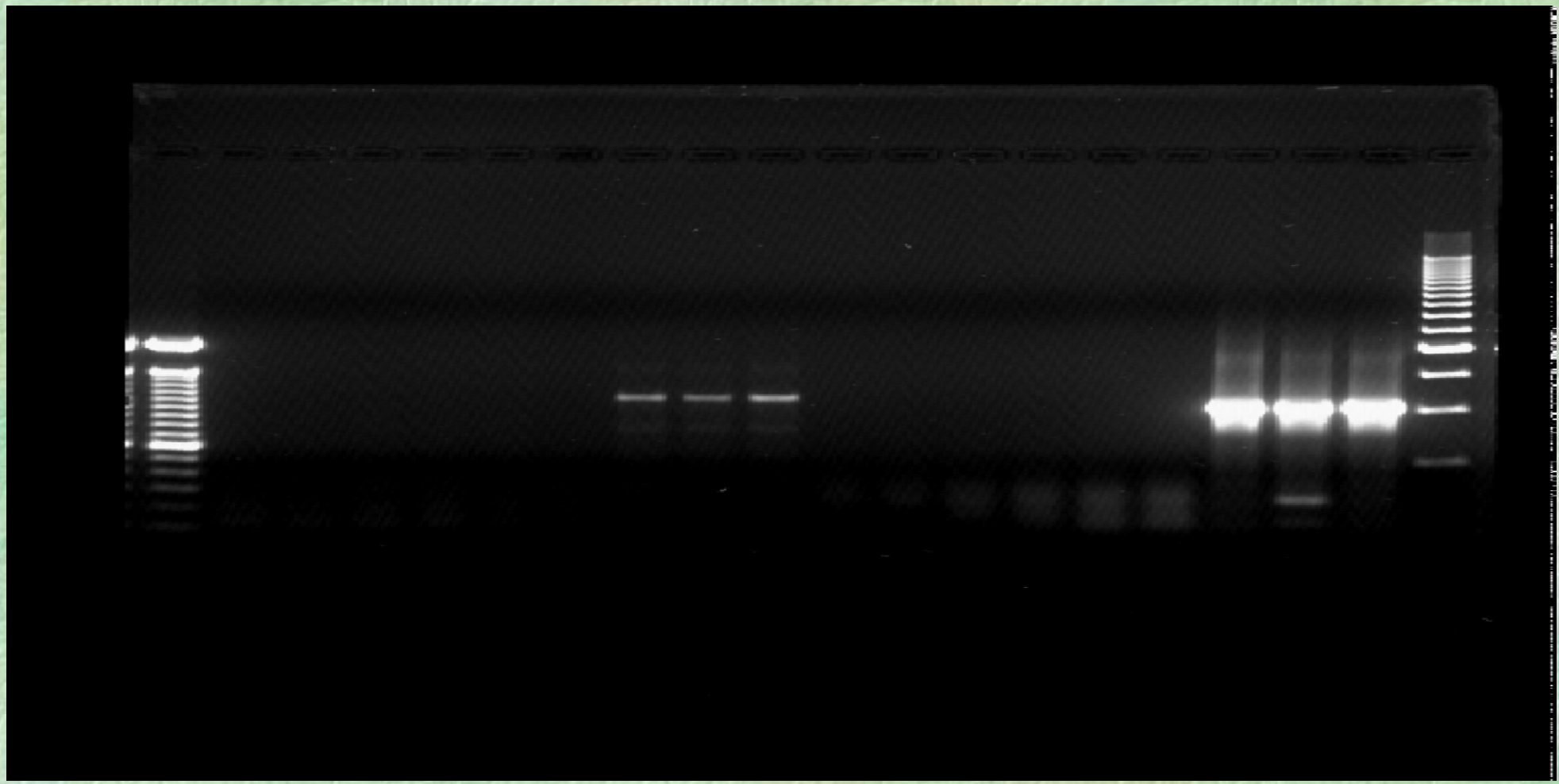
Effect of [$MgCl_2$] on Amplification

1.5 mM $MgCl_2$ 3.0 mM $MgCl_2$



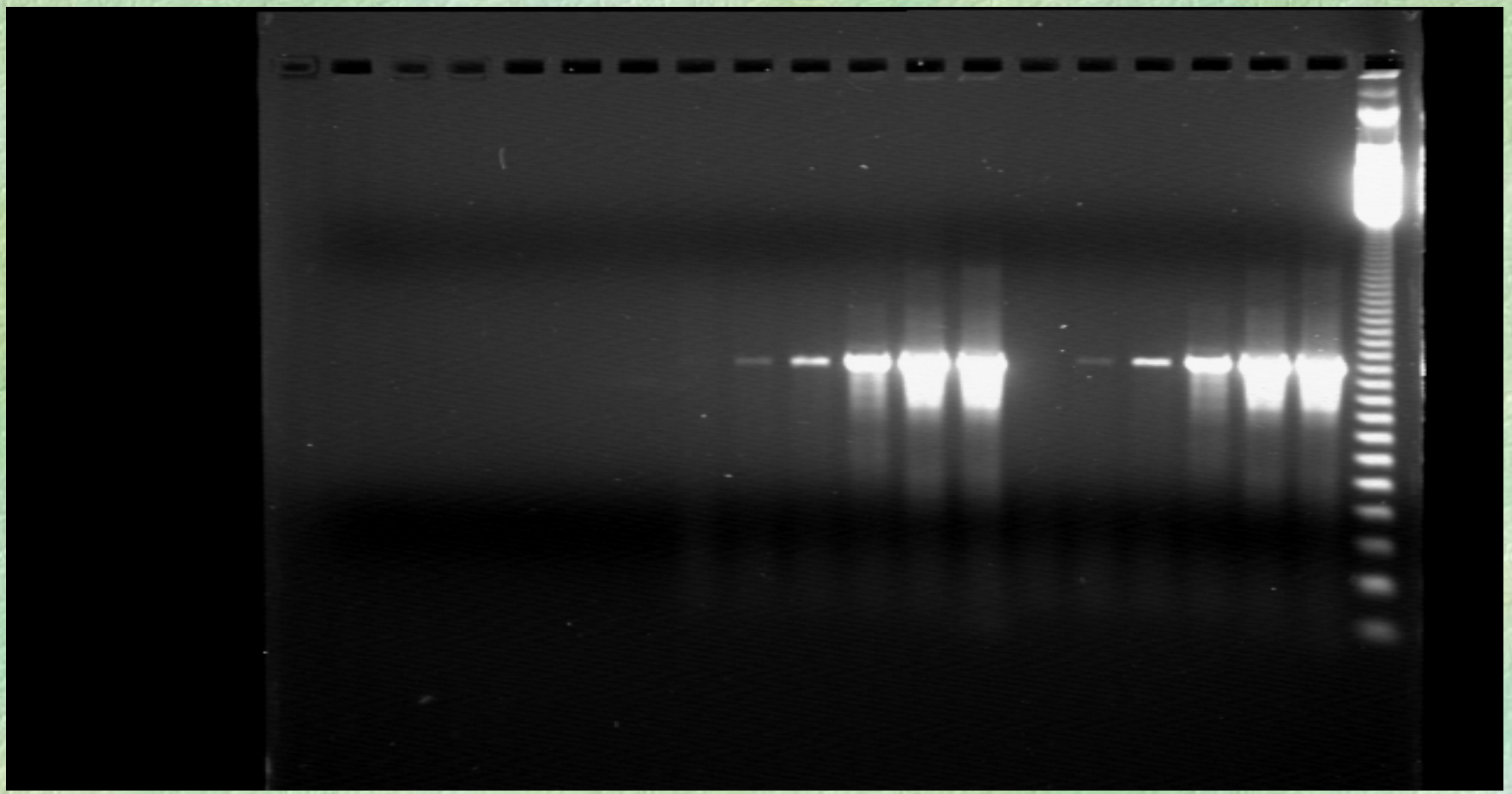
Annealing Temperature = 60 ^oC

Optimized SCAR Reaction Conditions (1.5 mM $MgCl_2$; 70°C Annealing)



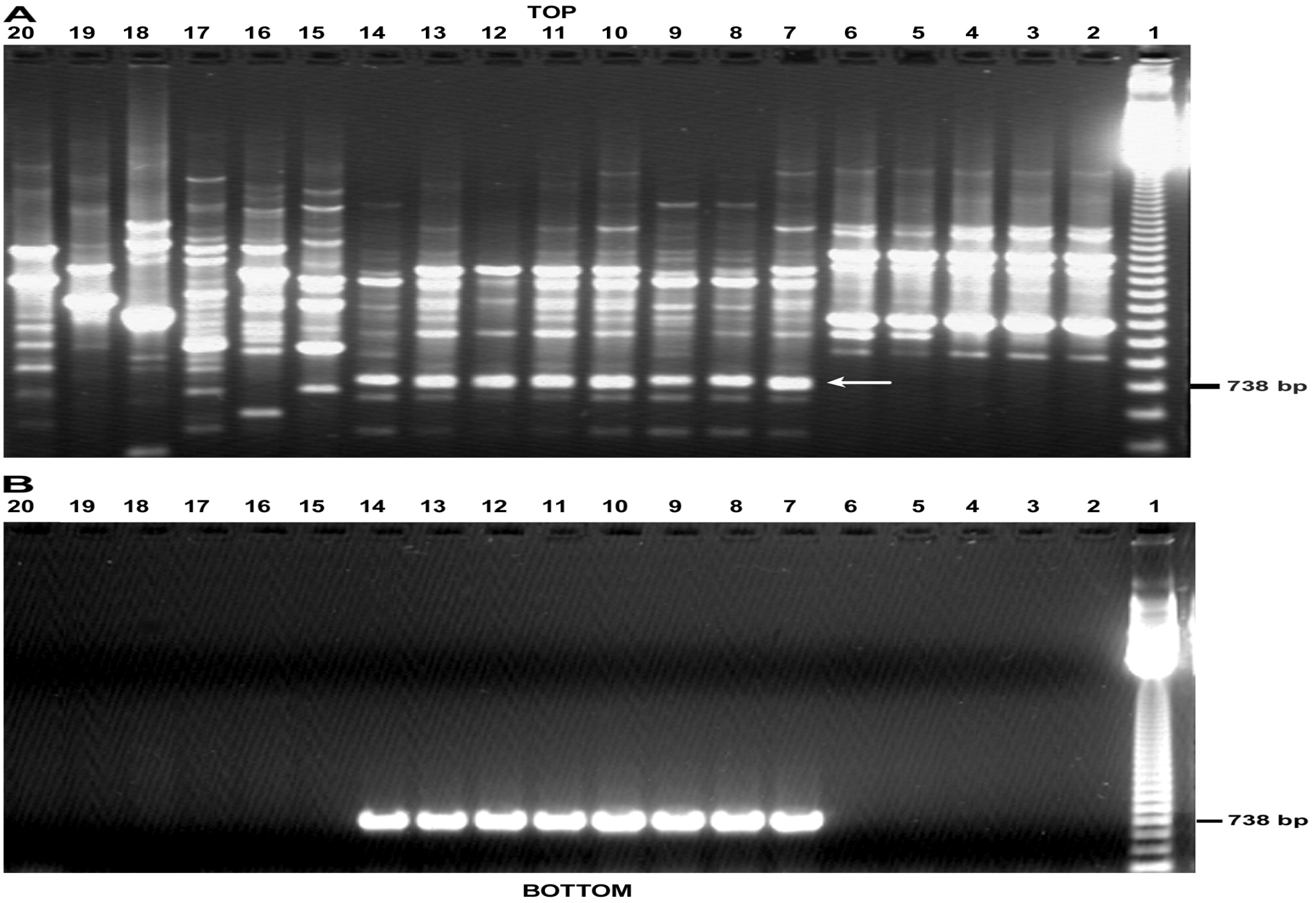
Effect of Cycle Number on Amplification[1]

20 40 20 40

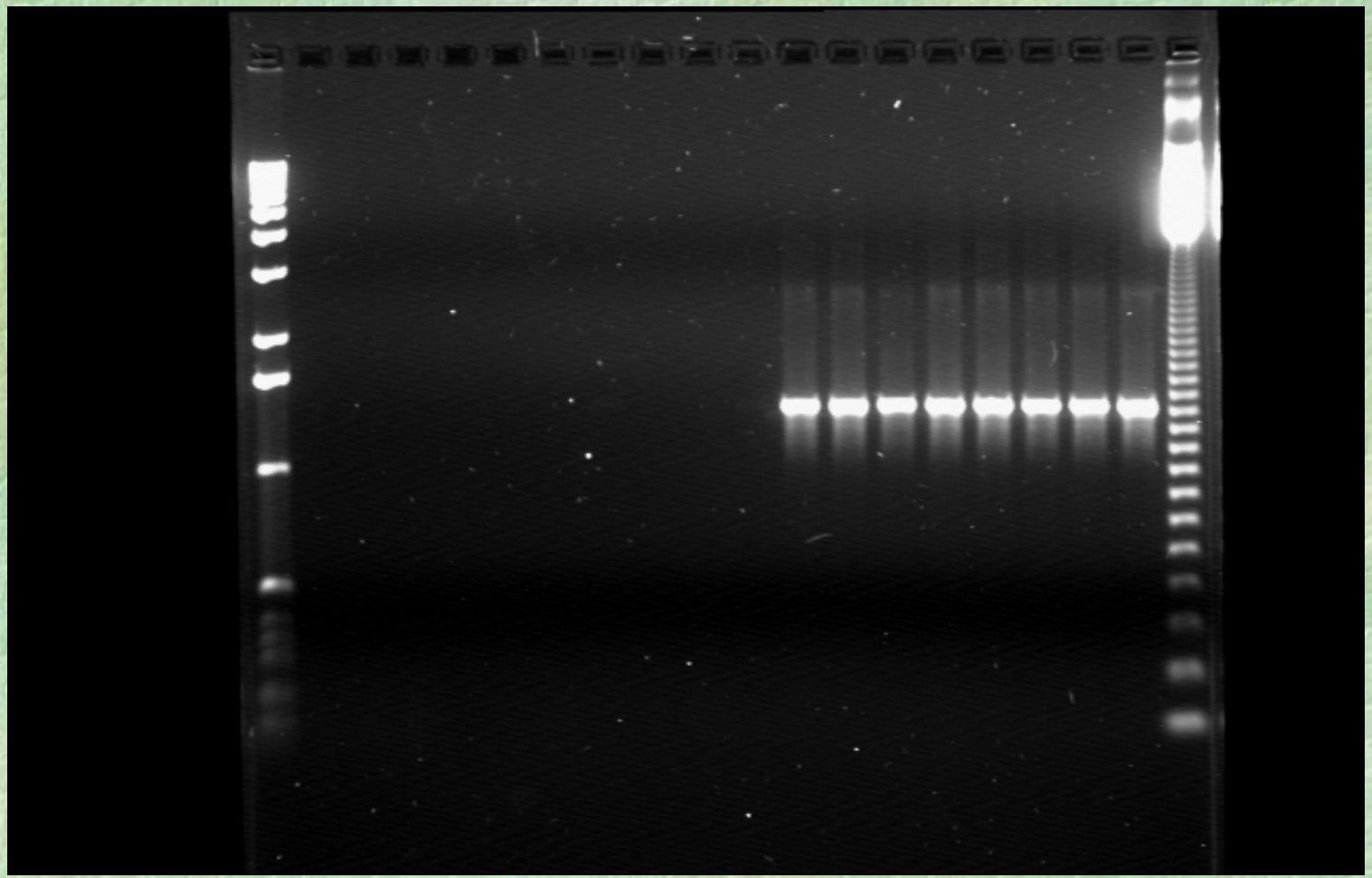


[1]Two-Step PCR {94°C (1 min) ↔ 72°C (1 min)}

Developing a SCAR Specific for *A. cochlioides*



A Single PCR Product (SCAR) is Diagnostic of *Aphanomyces euteiches*

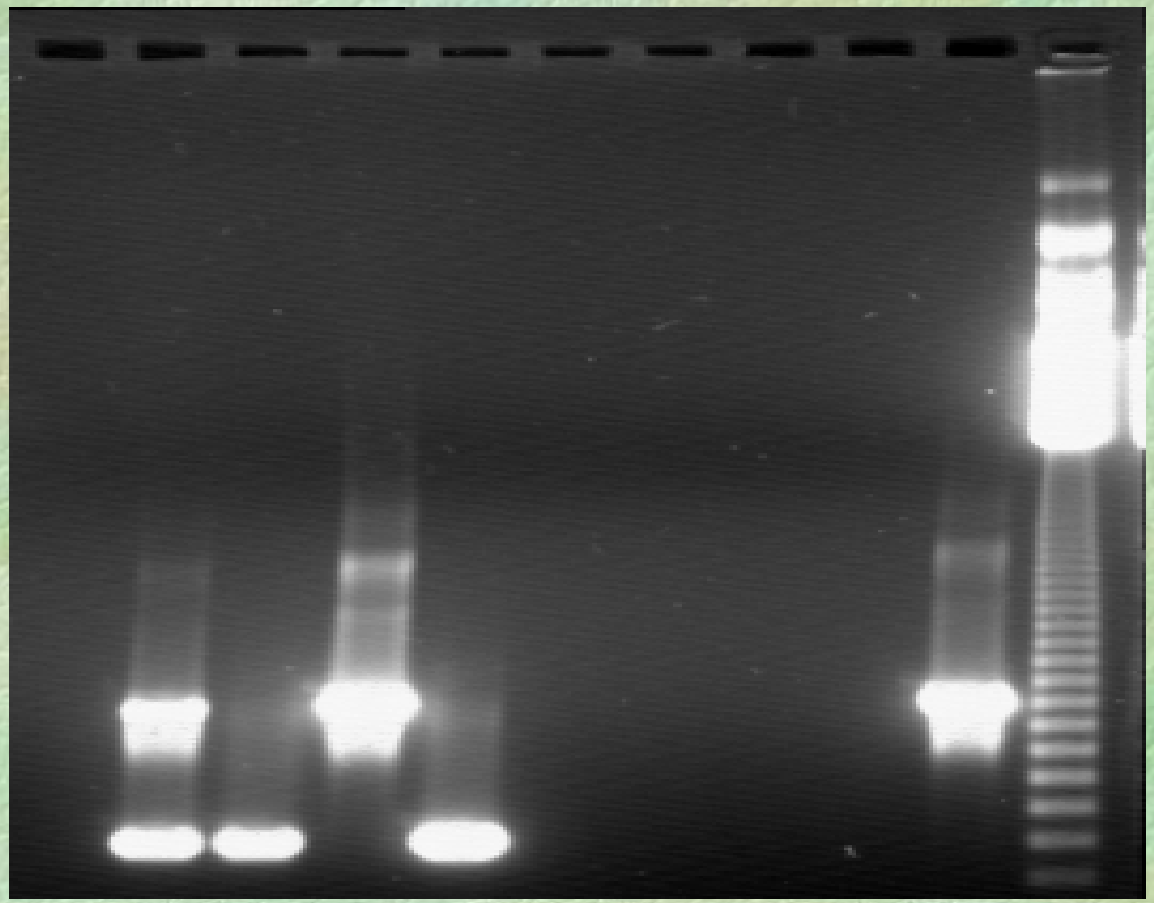


Soil microbes tested with SCARs

Aphanomyces euteiches	21
Aphanomyces cochlioides	8
Phytopthora infestans	2
Pythium ultimum	3
Pythium aphanidermatum	1
Pythium dissoticum	1
Fusarium oxysporum	4
Fusarium solani	2
Thelaviopisis basicola	2
Rhizoctonia solani	2
Mycosphaerella pinodes	1
Achlya spp.	6

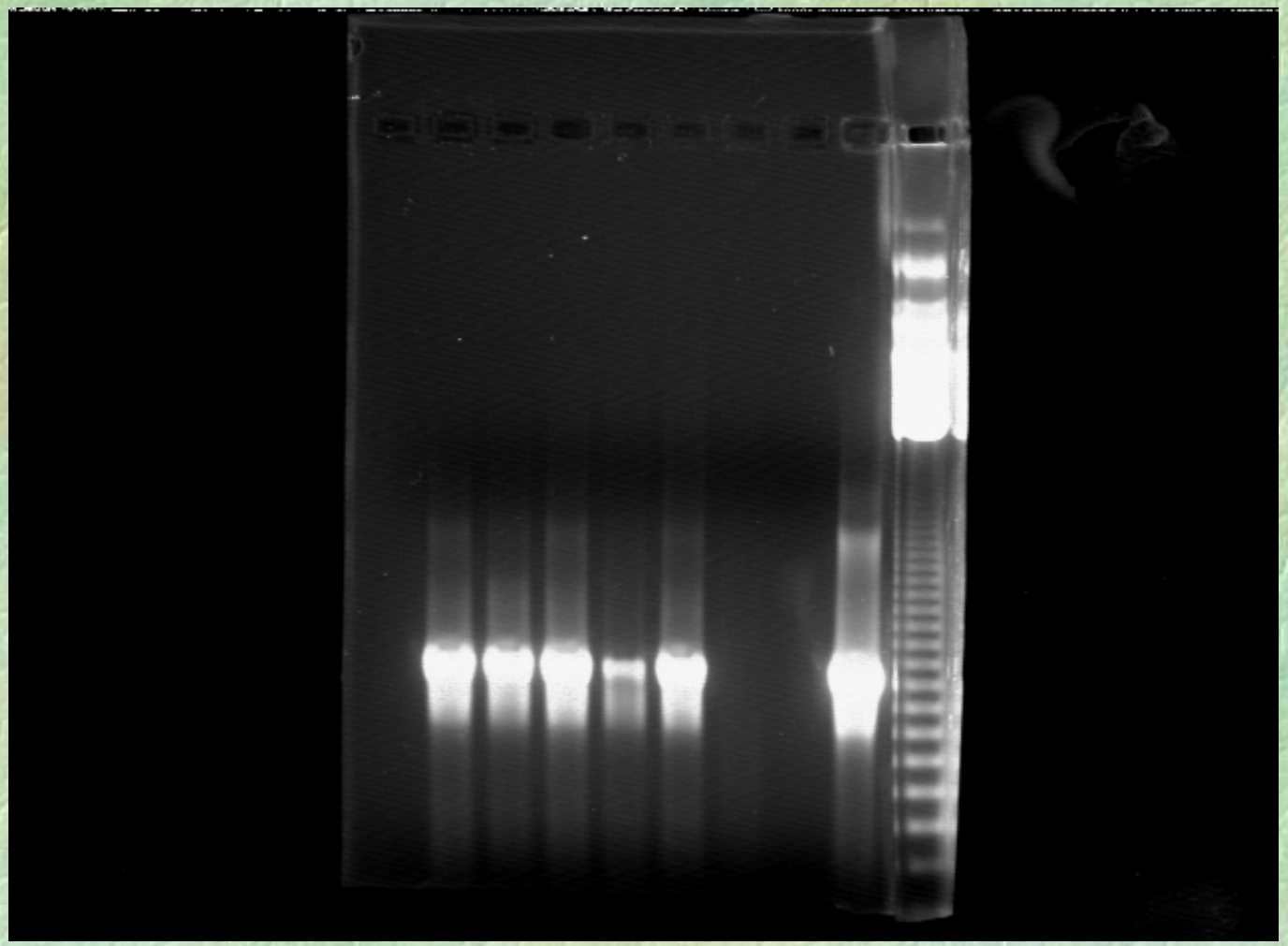
Multiplex PCR Demonstrates Species-specific Nature of SCAR Primers

Mixture *A. cochlioides* *A. euteiches*

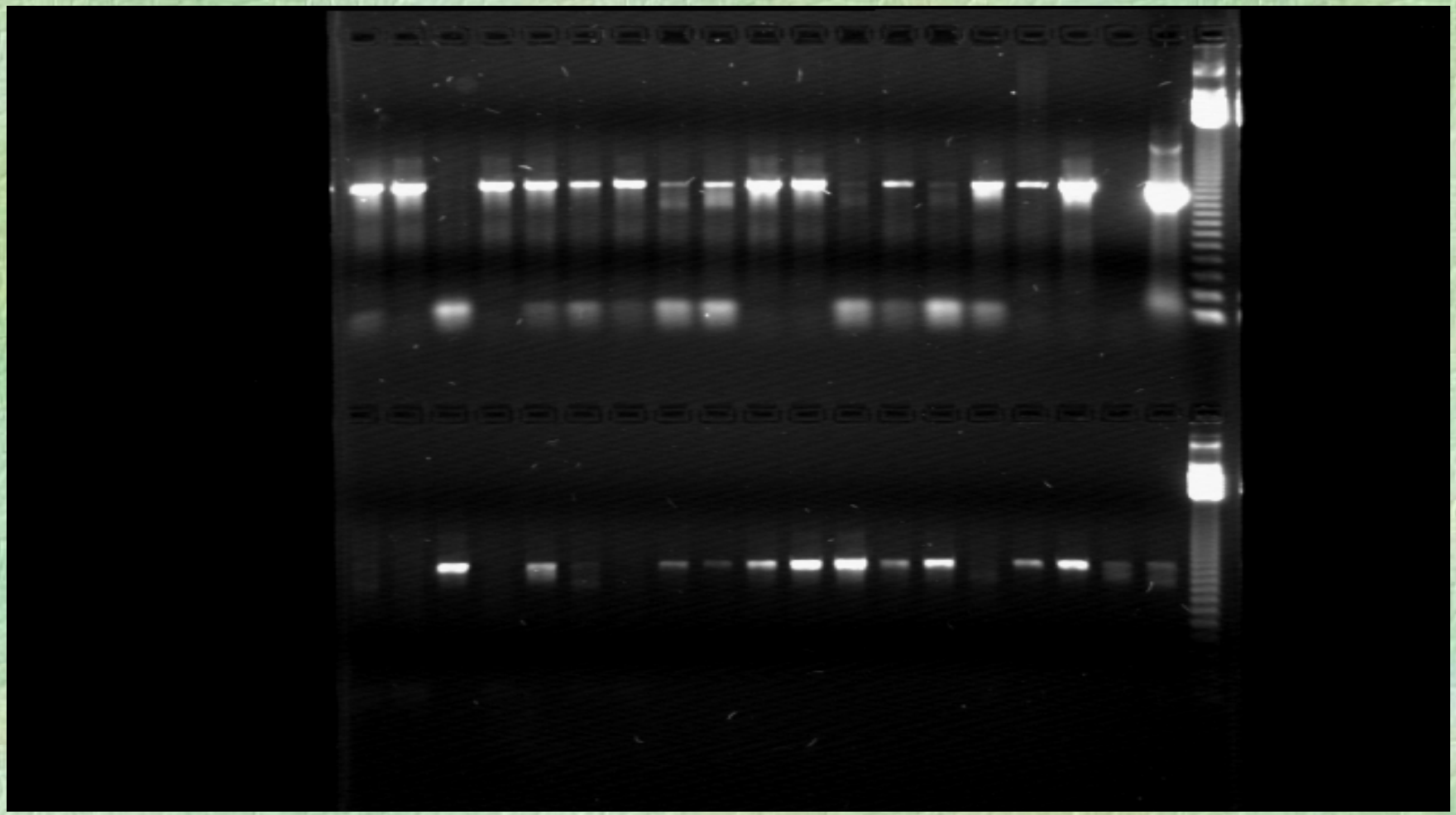


SCAR Primers can Detect *A. euteiches* in Infected Roots

Infected roots H A. e



Detection of A. euteiches in Field Grown Plants



SCAR Primers can Detect *A. euteiches* in Organic Debris Fraction of Field Soil

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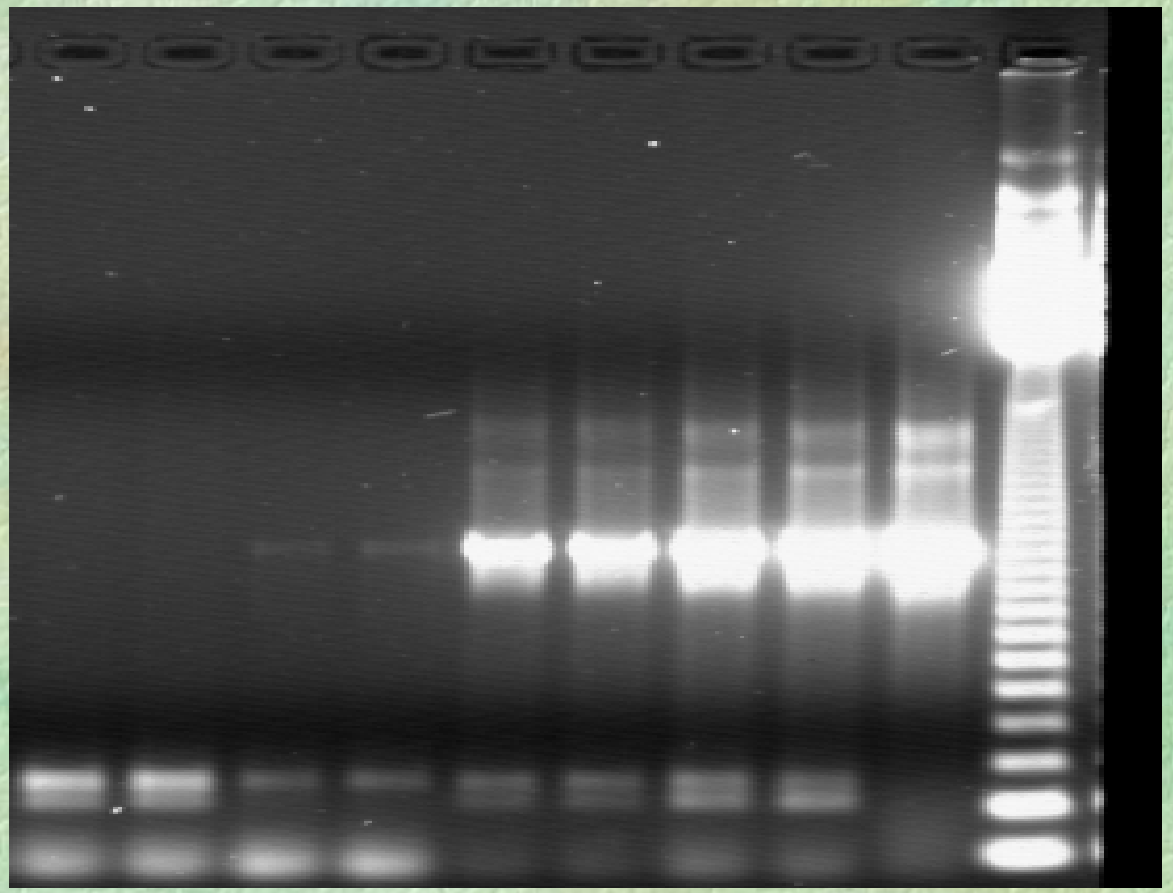

Possible Applications for SCARs

- Qualitatitive
  - Detection of pathogen in soil samples
  - Detection of pathogen in infected tissue
  - Discriminate between *Aphanomyces* spp.
- Quantitative
  - Compare pathogen colonization/movement between different plant genotypes.
  - Indirect selection for resistance among heterogeneous populations